

# The National Marine Biological Analytical Quality Control Scheme

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Macroalgae Component - Algal Identification Module Report – RM RT08 2014

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# MACROALGAL IDENTIFICATION MODULE REPORT FROM THE CONTRACTOR SCHEME OPERATION -2013-14

1	Introd	Introduction			
	1.1 Si	ummary of Performance	2		
2	Summ	ary of Macroalgae Component	3		
	2.1 In	ntroduction	3		
	2.2.1	Logistics	3		
	2.2.2	Analysis and Data Submissions	3		
	2.2.3	Confidentiality	3		
	2.3 N	Macroalgae Ring Test (RM RT07) Module	3		
	2.3.1	Description	3		
	2.3.1.1	1 Preparation of the Sample	4		
	2.3.1.2	2 Analysis Required	4		
	2.3.2	Results	4		
	2.3.2.1	1 General Comments	4		
	2.3.2.2	2 Analysis and Scoring of Data Returns	4		
	2.3.2.3	Ring Test Results	5		
	2.4 D	viscussion	6		
3	Conclu	usions and Recommendations	8		
◢.	Refere	onces	9		

#### 1 Introduction

To enable correct water quality classification and good management decision-making, quality control of biological data is a high priority. This extends through all biological elements including macroalgae and seagrass. Good quality control ensures consistency of data being reported for management purposes, and for macroalgae and marine angiosperms this has been driven primarily by the requirements of the Water Framework Directive. This QC scheme aims to facilitate improvements in biological assessment whilst maintaining the standard of marine biological data. The scheme should help to ensure consistency between analysts with improved confidence in ecological quality status.

The National Marine Biological Analytical Quality Control (NMBAQC) Scheme addresses several issues relating to macroalgae and seagrass data, this report focuses on one of these:

## The identification of macroalgae species

This is the eighth year in which the identification of intertidal macroalgae has been included as an element of the NMBAQC scheme, with the format following that of previous years. Test material was labelled and distributed to participating laboratories using previously employed procedures, from which species identification forms were completed and returned for analysis.

Eight laboratories subscribed to the macroalgae ring test with six laboratories submitting results with a total of eleven participants. Two laboratories failed to submit results; no reasons were provided. Four of the subscribing laboratories were government organisations and two were private consultancies. To ensure consistency between scheme years, each participating laboratory was assigned the same laboratory code as in previous years except where a laboratory was new to the scheme. Individual codes may, however, change slightly due to variations in individual participants. Due to the nature of the exercise there was no limit on the number of participants per lab.

Currently this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be treated as training exercises. However, a pass rate of 80% is suggested as an indicator of good performance, which may be used by competent monitoring authorities for internal monitoring of performance. Ring tests offer a means of assessing personal and laboratory performance from which continued training requirements may be identified or from which improvements in current field and laboratory procedures may be addressed.

# 1.1 Summary of Performance.

This report presents the findings of the macroalgae identification component for the eighth year of operation within the National Marine Biological Analytical Quality Control (NMBAQC) Scheme. This component consisted of a single macroalgae exercise the analytical procedures of which remained consistent with round seven of the scheme (RM RT07). The results for the exercise are presented and discussed with comments provided on the overall participant performance.

Images of twenty macroalgae specimens were distributed to the eight subscribing laboratories. Round eight of the ring test produced an acceptable degree of agreement between identifications made by participating laboratories and initial identification as made by Wells Marine. However, the ring test incorporated more challenging species than in previous tests resulting in a greater number of misidentifications than seen in earlier years.

# 2 Summary of Macroalgae Component

#### 2.1 Introduction

There was one module for the macroalgae identification component for scheme year eight. This module is described in full below to include details of distribution and logistics, completion of test result forms and full analysis and comparison of final submitted results.

# 2.2.1 Logistics

The test material was distributed on CD to each laboratory with labelling and distribution procedures following those of previous years. Each disc contained the full identification module including photos and habitat details from which to identify specimens, description of methods and data submission forms. Participants were primarily given six weeks to complete the test and return the results. However this was extended to 8 weeks due to late submissions and requests for extensions. There were no restrictions on the number of participants per laboratory.

Email has been the primary means of communication for all participating laboratories subsequent to the initial postal distribution of test material.

# 2.2.2 Analysis and Data Submissions

A prepared results sheet was distributed with the exercise instructions to standardise the format in which the results were submitted as per previous years. All returned data was done so in Excel and has been stored and analysed in this format. In this and previous scheme years slow or missing returns for exercises lead to delays in data processing data, reporting and feedback of results, therefore reminders were distributed shortly before the exercise deadline.

# 2.2.3 Confidentiality

To preserve the confidentiality of participating laboratories, each participant is allocated a four digit laboratory code from which they can identify their results. These codes are randomly assigned. The initial letters (MA) refer to the scheme this is followed by the scheme year which refers to the year in which the NMBAQC scheme original commenced, the final two digits represent the laboratory. For those laboratories where multiple submissions were provided the four digit code is followed by a letter allocated to each participant of that laboratory. For example, participant c from laboratory twelve in scheme year twenty one will be recorded as MA2112c.

# 2.3 Macroalgae Ring Test (RM RT08) Module

#### 2.3.1 Description

This training module enables the inter-laboratory comparisons of participants' ability to correctly identify macroalgae taxa and whether errors may be attributed to inadequate keys, lack of reference material or incorrect use of satisfactory keys.

One set of photographs for twenty specimens was distributed in January 2014. The specimens included a range of Chlorophyta, Rhodophyta and Phaeophyta and a mix of macroscopic and microscopic specimens from a variety of habitats including epilithic, epiphytic and endozoic species. There were a number of photographs per taxon showing different aspects of the alga and its habitat. Some supplementary information on habitat was included.

# 2.3.1.1 Preparation of the Sample

Each specimen was to be identified through a number of in-situ, macroscopic and microscopic photographs. In total a minimum of five photographs was used for each specimen collected by Wells Marine for the purpose of this exercise. Specimen photographs were obtained from a range of surveys from around the coast of the UK. Photographs were selected to represent sufficiently each specimen including in-situ (where possible), overall structure, branching patterns, cellular arrangements and cell contents making sure to include key characteristics for accurate identification. Scale bars were included where appropriate. Attempts were also made to ensure a high quality of photographs primarily focusing on clean specimens with sharp photographs.

Using a photographic test is considered a more practical means of testing macroalgal identification skills than preserved samples. These are known to lose colour rapidly and cell contents may become distorted making key characteristics more difficult to distinguish. Equally, fresh samples would not last a sufficient period to enable identification. It may also be difficult to obtain sufficient numbers of more unusual taxa for distribution to all laboratories.

# 2.3.1.2 Analysis Required

The participating laboratories were required to identify each of the macroalgae specimens from the photographs provided. Additional information should also be submitted including brief notes, information on keys used or possible problems with identification or quality of photograph provided. Expressing the level of confidence of identification should also be detailed, as this can aid in results of any disputes and in the preparation of reports. Participating laboratories were permitted to submit multiple data entries for each exercise to maximise results and allow sufficient comparisons of data entries. The protocol for circulating and completing the module followed that of previous years with six weeks (later extended to 8 weeks) allowed for the identification and submission of results.

#### 2.3.2 Results

## 2.3.2.1 General Comments

The scheme has taken on the same format as previous years; this includes the format of the test and method of data analysis and scoring. The macroalgae ring test can act as a training aid in the identification of species allowing those difficult taxa to be revealed and further identifying problematic areas.

For this current round of the scheme (RM RT08) specimen photographs were circulated to a total of eight laboratories. Six of the eight laboratories returned data entries with a total of eleven individual data sets.

Results were distributed to each of the participating laboratories four weeks after data submission. These results are documented in the preliminary results bulletin (RM RT08) which detailed individual scores and highlighted incorrect identifications, miss-spellings and use of synonyms. The bulletin also outlined reasons for identification discrepancies by comparing incorrect species and genus names with those of the AQC with the aid of photographs to pick out key characteristics.

#### 2.3.2.2 Analysis and Scoring of Data Returns

Laboratories returned lists of their species identifications within the format provided; these were compared against AQC identification as determined by Wells Marine to assess the number of

differences. The method of data comparison was achieved by comparing both the genus and species names and identifying where these differed with the AQC names. Such comparison included differences in spelling or use of a valid synonym for example:

- Use of different synonym for a taxon, e.g. Gelidium sesquipedale for Gelidium corneum
- Mis-spelling of taxa name, e.g. Rhodomela lycopoides for Rhodomela lycopodioides

Such differences were highlighted, but not taken into account during calculation of the total number of differences in identification.

Data entries were tabulated (as seen in RM RT08 Preliminary Results Bulletin, Table 2) in order of specimen number and laboratory. The individuals' data entries are only given where they differ from the AQC identification. This includes those entries for which species are spelled incorrectly or where an appropriate synonym is provided as well as those instances in which the specimen has been identified incorrectly. For those entries in which a synonym or mis-spelling was supplied by the participant but for which the identification was consistent with that of the AQC, the name was presented in brackets [species name]. Those entries in which the identification was considered different to the AQC the species or genus name that did not correspond to the AQC was provided in the table. If part or the entire species name entered was correct this was indicated by a dash "-" any incorrect name was included in the table e.g. where Gelidium sesquipedale was identified as Gelidium latifolium this would be entered as " – latifolium". Further exceptions were granted to Gelidium sesquipedale. Due to the lack of distinguishing features between Gelidium sesquipedale and Gelidium pulchellum and inadequate keys to clearly distinguish these species both were accepted for the identification of species RT0817.

The data entries for an individual were scored by one where the entry was consistent with that of the AQC. For instance where text other than a dash "-" or a bracketed name [name] is provided no score was given. This includes differences at both genus and species level, although species can be considered a largely independent value (where the generic identification was incorrect then the species identification would also be incorrect). Therefore where the full genus and species name was correct a score of two would be given; where either genus or species name was incorrect a score of one would be given. The method of scoring applied to those species in which a correct identification was provided and included those instances where synonyms were used or species/genus names spelled incorrectly.

# 2.3.2.3 Ring Test Results

RM RT08 contained twenty specimens for identification for which there was a good general level of agreement through all eleven participants. At the generic level there were a total of forty differences (from a potential two hundred and twenty) across the eleven sets of data received from the six participating laboratories (18.18%). At the specific level there were a total of sixty eight differences (30.91%), which is higher than the previous years' results. These differences could be attributed primarily to just a few taxa. A total of 20% of all errors were from one species (*Boergeseniella thuyoides*) contributing to 23% of all generic differences and 16% of all specific differences. *Desmarestia viridis, Petalonia fascia* and *Gelidium corneum* contributed to a further 14%, 14% and 13%, respectively, of differences.

A further six specimens contributed individually between 6% and 9% of both generic and specific differences attributing to 44% of overall errors (*Elachista flaccida, Eudesme virescens Delesseria sanguinea, Osmundea osmunda, Ceramium gaditanum* and *Ulothrix flacca*). A final two taxa were

responsible for the remaining two specific and one generic error (*Asperococcus fistulosus* and *Dumontia contorta*). The remaining eight species received no generic or specific identification errors.

The difference between participants' entries and AQC identifications was generally well distributed with all participants identifying at least one species incorrectly and only one lab (MA2103e) correctly identifying all genera. The overall scores and number of incorrect identifications ranged from two to fifteen with no one participant identifying all genera and species correctly. At this stage the levels of low, medium and high scoring have not been established for this particular ring test so participants and laboratories cannot be allocated a level of acceptance based on their overall score.

# 2.4 Discussion

This is the eighth macroalgae identification ring test as circulated through the NMBAQC scheme, with early exercises being essentially trials of the methodology. Although the results were broadly comparable with those of previous years (RT06 and RT07) there is a noticeable decrease in the level of agreement between participating laboratories and the AQC. As per last year's suggestions an increased number of cryptic and taxonomically challenging species were included in the test. Such genera included *Gelidium*, *Ulothrix* and *Ceramium* which can be difficult to identify to species level. These genera require an increased depth of knowledge on the cellular attributes and other characteristics, which can be remarkably similar between species. As intended by the scheme these tests aim to challenge participants and assist with training by stimulating the use of various keys and increasing familiarity with taxonomic terminology. Further, it allows problem taxa to be identified stimulating areas for inclusion in workshops, and targeting such taxa within future exercises. Photographs used within the ring tests may be retained within the participating laboratories for future reference, with some descriptions allowing the comparison of taxonomically similar species.

No laboratory or individual managed to identify all species and genera correctly and there were only 8 species (40%) for which all laboratories were successful in their identification (Table 1 and Figure 1). The most problematic species was *Boergeseniella thuyoides* which may be considered relatively difficult to identify due to the occurrence of morphologically similar species. Those characteristics which are considered more specific and may be used to distinguish such species were detailed within the Bulletin. Although the largest portion of incorrect identifications could be attributed to a few species, there was equally a high number of species for which at least one laboratory identified incorrectly; however, there were no specimens which were not identified successfully by at least one laboratory.

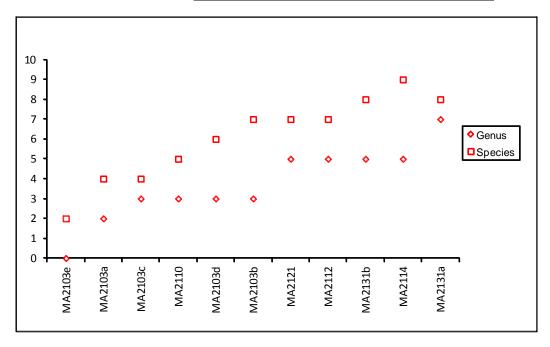
Another issue arose with *Gelidium sesquipedale*. This is a fairly common species, but with limited distributed and highly variable morphologically. Similar species have overlapping characteristics, and it was considered that this overlap between *G. sesquipedale* and *G. pulchellum* was sufficient to justify accepting both names on this occasion. In this instance it was also unclear which keys or guides were used to identify the species making them impossible to compare. This information is vital to determine if the guide descriptions were insufficient to correctly identify the species or if the photographs provided were insufficient. Additionally, it is recognised that some keys require revision, but this is not within the scope of NMBAQC.

At this time the use of a photographic test is considered the most effective means of testing macroalgal identification skills. Preserved samples are known to rapidly to lose colour with cells becoming distorted making key characteristics more difficult to distinguish. Equally, fresh samples would not last a sufficient period to enable identification. However, it is possible that some photographs were not considered to be of sufficient quality to correctly identify the specimens

despite all efforts. This may have attributed to some confusion over the identification of some more cryptic species.

**Table 1:** Summary of differences in identification.

			Total differences for 11 returns	
Specimen	Genera	Species	Genus	Species
RT0801	Desmarestia	viridis	6	8
RT0802	Ulva	rigida	0	0
RT0803	Cystoclonium	purpureum	0	0
RT0804	Elachista	flaccida	3	3
RT0805	Eudesme	virescens	3	4
RT0806	Mastocarpus	stellatus	0	0
RT0807	Ascophyllum	nodosum	0	0
RT0808	Bryopsis	plumosa	0	0
RT0809	Delesseria	sanguinea	4	4
RT0810	Osmundea	osmunda	0	8
RT0811	Asperococcus	fistulosus	0	1
RT0812	Ceramium	gaditanum	0	6
RT0813	Laminaria	hyperborea	0	0
RT0814	Boergeseniella	thyoides	9	11
RT0815	Dumontia	contorta	1	1
RT0816	Petalonia	fascia	7	7
RT0817	Gelidium	corneum	6	7
RT0818	Spongonema	tomentosum	0	0
RT0819	Ulothrix	flacca	1	8
RT0820	Gastroclonium	ovatum	0	0
		Total differences	40	68
		Average differences per Genus/species	2.000	3.400



**Figure 1:** The number of differences from the AQC identification of intertidal macroalgae specimens, for each of the participating laboratories for RT08, arranged in order of increasing number of differences.

# 3 Conclusions and Recommendations

- The seventh macroalgae ring test exercise was implemented successfully and completed by most
  participants with a general agreement of the format. All feedback has been reviewed and will be
  considered for subsequent exercises; such feedback is encouraged to enable the protocols to be
  refined.
- 2. The relatively good level of agreement within this test provides evidence that macroalgae identification skills are increasing; however there are still a number of problematic areas. This is to be expected, as some taxa are inherently more difficult than others. The errors occurring were generally at the specific level, however where generic errors occurred these were most often with taxonomically similar species which share similar characteristics and are therefore hard to separate. Such species will be noted for possible future workshops and will be targeted in future exercises.
- 3. There were still a number of incorrect spellings; therefore participants are urged to take more care prior to submitting results to ensure all names are spelled correctly. This is equally important when submitting data records or reports where scientific names are incorporated. It should also be noted that a number of data spreadsheets were not fully completed, often missing out the keys or guides that were used. This may seem trivial information but can help identify where the participant has been mislead with the keys or help explain how or why an alternative identification was reached. For future ring tests it is requested that the data spreadsheets be completed in full, including level of confidence in the identification Participants should include the authority alongside taxon names, as this also aids in the analysis of returns.
- 4. As with some previous tests there was some disagreement as to the correct identification of some species. Descriptions of some species have recently changed; some have resulted in nomenclatural changes or use of more specific characteristics that were previously considered more generic. New studies in species taxonomy are regularly highlighting previously unidentified (cryptic) species, splitting one species into two based on a previously unknown characteristic. In these instances both species identification have been accepted such as *Gelidium sesquipedale* and *G. pulchellum*. Keying out the two species shows very little difference except for some basic morphological differences, or at the microscopic level which was not fully evident through the photos provided. This problem highlights the need for more definitive photos, specimens and descriptions to be provided in future exercises so as to save confusion.
- 5. All laboratories are encouraged to keep all test photographs within a reference collection. This has a number of benefits particularly with regards to improving identification ability, training new staff and maintaining consistency of identification between surveys and staff. This reference collection should also be extended through to literature to ensure current keys are used with up to date nomenclature. A list of identification works will be given on the NMBAQC website. However, this is not exhaustive, and does not necessarily include unpublished keys provided at workshops unless specifically authorised by the key's author.
- 6. During this eighth cycle of the macroalgae identification exercise all participants' submitted results within the designated (extended) timescale. In future exercises all laboratories should

- continue to submit results within the requested deadlines as detailed at the beginning of the exercise. In subsequent years reminders will continue to be distributed two weeks prior to the completion of the exercise.
- 7. There are still some issues over the timing of the test and there are suggestions that the time allowed for completion of the test should be extended to accommodate increased workloads. Despite some problems with the timing of the test and suggestions of it being held in October/November, a large majority of responses indicated that early in the year is still the most appropriate time for the tests. However the tests shall now be distributed within the first week of January. The time scale in which to complete the test shall remain at 6 weeks.
- 8. Although there was general approval on the quality, detail and use of photographs with most participants agreeing on the levels of difficulty, there were some areas which require some improvement. In some instances the *in situ* specimen photographs would have benefitted further from a scale and additional details of habitat, general location, exposure of shore, height present on shore, etc than were provided. This additional information will be included in subsequent tests to allow more accurate identification and to reduce error or confusion. Some more specific cellular information was also requested within the photos, and where possible this will be achieved such as cross sections of filamentous species such as *Ceramium* or *Polysiphonia*. However, even when looking at fresh specimens not all such characteristics may be present, e.g. reproductive structures. No staining is currently used and this shall remain for the following test. All attempts will be made in the future to ensure that sufficient material is provided, allowing correct identification to species level.

If anyone has further comments on this, or disagrees with any of the interpretation, please pass forward your comments to Dr Emma Wells (<a href="mailto:emma@wellsmarine.org">emma@wellsmarine.org</a>) or Dr Clare Scanlan (<a href="mailto:clare.scanlan@sepa.org.uk">clare.scanlan@sepa.org.uk</a>). This ring test is continually being refined to ensure it provides the best opportunity to test macroalgae identification skills so all suggestions and comments are welcomed.

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